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ADDITIONAL VALUE OF
CSF A β 40 LEVELS IN THE
DIFFERENTIATION BETWEEN
FTLD AND CONTROL SUBJECTS

3.2



ABSTRACT

To determine the additional value of cerebrospinal fluid (CSF) amyloid beta 1-40 (A β 40) next to amyloid-beta 1-42 (A β 42), total tau (tau) and tau phosphorylated at threonine-181 (ptau-181) to distinguish patients with frontotemporal lobar degeneration (FTLD), Alzheimer's disease (AD), and controls, we measured CSF levels of A β 40, A β 42, tau and ptau-181 in 55 patients. Logistic regression was used to identify biomarkers that best distinguished the groups. Additionally, a decision tree (cost = test method; Matlab 7.7) was used to predict diagnosis selecting the best set of biomarkers with the optimal cut-off. Logistic regression showed that A β 42 and ptau-181 CSF levels provided optimal distinction between AD and FTLD. A combination of A β 42, tau and A β 40 optimally discriminated FTLD from controls, and AD from controls. The decision tree used A β 42 (cut-off 578 pg/ml) to identify AD (positive predictive value (PPV) 97%), followed by tau (cut-off 336 pg/ml) to identify FTLD (PPV 67%) and in the last step A β 40 (cut-off 10 ng/ml) was used to differentiate controls (PPV 68%). Applying CSF A β 40 levels in the model, the PPV of diagnosis increased to 75% as opposed to 70% when only A β 42 and tau were used. CSF A β 40 levels added to the conventional CSF biomarkers increases the potential to discriminate subjects with dementia from controls. Our findings favor the implementation of CSF A β 40 CSF in differential diagnosis between FTLD, AD and controls.

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INTRODUCTION

Frontotemporal lobar degeneration (FTLD) is a spectrum of neurodegenerative disorders affecting the frontal and/or temporal lobes. Its three prototypical clinical variants are the behavioral variant frontotemporal dementia (bvFTD), and the language variants semantic dementia (SD) and progressive non-fluent aphasia (PNFA).¹ FTLD is pathologically heterogeneous, and can be grossly divided into tau-related pathologies and TAR DNA binding protein (TDP-43) related pathologies.² FTLD is commonly mistaken for Alzheimer's disease (AD) or primary psychiatric disorders.³

Cerebrospinal fluid (CSF) biomarkers may serve as an aid in clinical diagnosis as they are thought to reflect pathological processes taking place in the brain. Until now, no satisfactory set of biomarkers to distinguish FTLD from both AD and controls, has been found.⁴⁻¹⁵ CSF biomarkers have been found to be most valuable in the distinction between AD and non-demented subjects. Lower CSF levels of amyloid-beta 1-42 (A β 42) and higher CSF levels of total tau protein (tau) and its phosphorylated form (ptau) can discriminate AD patients from controls at a high sensitivity.¹⁶⁻¹⁸ However, the specificity of these biomarkers, is relatively lower, complicating their use in the differential diagnosis of dementia.¹⁶⁻¹⁸

In a preliminary study we found that CSF A β 40 (amyloid beta 1-40) levels were lower in FTLD patients compared with both AD patients and controls.¹⁰ The goal of this study was to investigate whether measurement of CSF A β 40 next to the conventional biomarkers A β 42, tau and ptau-181 has additional value in the discrimination between FTLD, AD and controls.

3.2

METHODS

Patients

Fifty-five patients with FTLD (36 patients with bvFTD, 14 with SD, and 5 with PNFA), 60 patients with probable AD and 40 control subjects were included in this study. All patients underwent a standardized clinical investigation including medical history, physical and neurological examination, screening laboratory tests, neuropsychological assessment, EEG and brain MRI. Clinical diagnosis was made by consensus in a multidisciplinary meeting without knowledge of CSF results. The diagnosis of AD was made using the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.¹⁹ For FTLD we used the international clinical diagnostic consensus criteria of Neary et al.¹ and the neuropsychological test used included the Visual Association Test (VAT) and the Rey auditory-verbal learning test for episodic memory, digit spans forwards and backwards for working memory and category fluency for semantic memory.²⁰⁻²² For executive dysfunction the following tests were used: the Trail Making Test (TMT) B, elements of the Behaviour Assessment of Dysexecutive Syndrome (BADS), the Stroop and letter fluency. The Boston naming test and VAT naming were used for naming and the Rey-Osterrieth Complex Figure Copying test for evaluating visuospatial function.^{23,24} The Mini-Mental State Examination (MMSE) was used to

evaluate the degree of cognitive impairment and the Clinical Dementia Rate (CDR) was used to assess the clinical state of the patients.²⁵

The control group consisted of 40 patients who presented at our memory clinic with subjective complaints, but who had normal clinical investigations, and did not have significant cognitive deficits. These patients with subjective memory complaints have been followed (mean 1.5 (1.4) year) and all control subjects remained stable at follow up, except one patient which was classified as MCI (follow up time 2.5 year). All patients and control subjects were enrolled at the memory clinic of the VUmc (Amsterdam, The Netherlands) between 1998 and 2007. The local ethical review board approved the study protocol and all subjects gave written informed consent.

CSF analysis

CSF samples were obtained by lumbar puncture between the L3/L4 or L4/L5 intervertebral space, 12 mL was collected in polypropylene tubes and within two hours brought to the lab. Part of the CSF was used for routine analysis, including total cell and erythrocyte count, as well as total protein determination. The remaining CSF was then centrifuged at 1800 x g for 10 minutes at 4°C. The CSF samples were aliquoted into polypropylene tubes and immediately stored at -80 °C until analysis. CSF A β 42, tau and ptau-181 were measured with Innostest sandwich ELISA as described previously.¹¹ As the manufacturer does not supply controls, the performance of the assays was monitored with pools of surplus CSF specimens. In the study period multiple specimens with various concentrations which were included in 7-18 runs have been used for this purpose. The inter-assay coefficient of variation (mean \pm SD) was 11.3 \pm 4.9% for A β 42, 9.3 \pm 1.5% for tau and 9.4 \pm 2.5% for ptau-181. The team of the department of Clinical Chemistry (VUmc) involved in the CSF analysis was not aware of the clinical diagnoses.

A β 40 was measured with two separate methods. One was a commercial ELISA (The Genetics Company, Zürich, Switzerland²⁶) and the other was our in-house assay. The intra-assay coefficient of variation (CV) of the Genetics Company method was 3.5% and the inter-assay CV 10.2%. The in-house assay is a sandwich ELISA employing an in-house produced monoclonal antibody against the COOH terminus at residue 40 (VU- α -A β 40) as catcher and biotinylated 6E10 (a commercially available monoclonal antibody against the NH2 terminus of the amyloid peptide; Signet laboratories, Dedham, MA, USA) as detecting antibody.²⁷ The intra-assay coefficient of variation (CV) was 1.4% and the inter-assay CV 7.3%.

Statistical analysis

Data were analyzed with the SPSS software package (version 15 for Windows SPSS, Chicago IL). In statistical analyses involving biomarker concentrations natural log-transformed concentrations were used, unless stated otherwise. Differences between groups were tested using Chi-squared test and analysis of variance (ANOVA) with sex and age as covariates. Bonferroni tests were used to adjust for multiple comparisons and for correlations the Pearson test was used. To identify the best subset of biomarkers for pair wise discrimination (FTLD versus AD, FTLD versus controls and AD versus controls), logistic regression analysis was used (forward likelihood; the entry of probability for stepwise analysis was set at $p < 0.01$). Sensitivity and specificity were derived from the models.

In an additional analysis, we performed tree regression (cost=test method; Matlab 7.7). Several variables and outcomes can simultaneously be entered in this model (in our case four biomarkers and three groups) to predict the outcome score on the basis of dichotomized variables. The model starts with determining the variable and dichotomization threshold that give the best prediction of the outcome score. Then, either the subset above or the subset below the threshold is split into two smaller subsets. This process is repeated and a prediction tree is constructed. The number of subsets (or branches of the tree) will be determined by minimizing the cross-validation prediction error. As a result the model predicts several decision steps that have to be taken (each time by use of just one variable) to optimally discriminate the groups from each other. Additionally, this model delivers the best cut-off value for each biomarker that has to be used for the consecutive different decisions.²⁸

RESULTS

The clinical characteristics and the biomarker levels by diagnostic groups are shown in Table 1. The FTLD, AD and control groups did not differ in sex. However, the three groups differed in age, as controls were slightly younger than AD patients ($p<0.05$). The MMSE score was lower in FTLD and AD patients than in controls (for both comparisons $p<0.01$). Moreover, the MMSE score was lower in AD patients than in FTLD patients ($p<0.01$). ANOVA revealed a significant group difference for all CSF biomarker levels (all $p\leq 0.01$). Post-hoc tests revealed higher levels of tau and ptau-181, and lower levels of A β 42 in AD compared with controls and FTLD patients (all $p<0.05$). Lower levels of A β 40 (both methods) and higher tau levels were found in FTLD compared with controls (both $p<0.05$).

3.2

Table 1. Clinical characteristics and levels of CSF biomarkers

	Controls (n=40)	FTLD (n=55)	AD (n=60)	p-value
Sex female (%)	19 (48%)	22 (40%)	33 (55%)	0.3
Age (years)	59 (10)	61 (7)	64 (8)	<0.05 ^b
MMSE	29 (1)	24 (5)	21 (5)	<0.01 ^{a, b, c}
CDR [median (min, max)]	0 (0, 0)	1 (0.5; 2)	1 (0.5; 2)	<0.01 ^{a, b}
A β 40, in-house method, ng/ml	12.3 (2.7)	10.6 (3.4)	11.0 (2.5)	0.01 ^a
A β 40 (genetics), ng/ml	9.4 (2.4)	7.9 (2.6)	8.4 (2.3)	0.01 ^a
A β 42 (Innogenetics), pg/ml	848 (200)	786 (296)	444 (139)	<0.01 ^{b, c}
Tau (Innogenetics), pg/ml	274 (125)	422 (289)	777 (353)	<0.01 ^{a, b, c}
Ptau-181 (Innogenetics), pg/ml	44 (15)	49 (25)	90 (31)	<0.01 ^{b, c}

Data are shown as mean (SD) or n (%). Please note that raw biomarker levels are shown, whereas statistical analyses were performed using natural log-transformed concentrations. MMSE score is known for 42 FTLD patients, 59 AD patients and 39 controls. CDR score is known for 53 FTLD patients, 57 AD patients and 40 controls. Post hoc results: ^a FTLD versus controls; ^b AD versus controls; ^c FTLD versus AD.

We calculated the mean biomarker values for each FTLD subgroup. All subgroups had no different biomarker levels except the PFNA subgroup (n=5) where a lower A β 42 (428 pg/ml) was found in comparison with SD (803 pg/ml) and bvFTD (830 pg/ml) (all $p < 0.05$). Comparison of the main groups (AD-FTLD-controls) and the post-hoc results did not change when the PFNA subgroup was excluded.

In Table 2 the results of logistic regression analysis are shown against the background of comparable CSF studies. A β 42 and ptau-181 were the best biomarkers subset to discriminate AD patients from FTLD patients (sensitivity 85% and specificity 87%). To discriminate FTLD from controls, levels of CSF A β 42, tau and A β 40 (in house method) were the best combination (sensitivity 86% and specificity 80%). Finally, to differentiate AD from controls A β 42, tau and A β 40, were the best subset of biomarkers (sensitivity 95% and specificity 95%). When A β 40 was omitted from logistic regression analysis to discriminate FTLD from controls, tau and ptau-181 were the best subset of biomarkers and sensitivity and specificity dropped to 76% and 68%, respectively. When this was performed for AD and controls, A β 42 and tau were the best subset of biomarkers and only the specificity dropped to 92.5%.

Subsequently four variables (A β 42, A β 40, tau and ptau-181) were entered in the regression tree model to predict the three diagnostic groups (Figure 1). In the first step, the model identified AD patients using CSF A β 42 levels at a cut off value of 578 pg/ml (positive predictive value (PPV) 97%). Subsequently, the FTLD group was selected using CSF tau levels (cut-off value 336 pg/ml). Finally, the control group (PPV 68 %) was separated from the FTLD group (PPV 67%) by use of CSF A β 40 levels (cut-off value 10 ng/ml for the in house method, 6 ng/ml for the commercial test). If only A β 42 and tau were used, the overall PPV was 70%. The PPV increased to 75%, when CSF A β 40 levels were included.

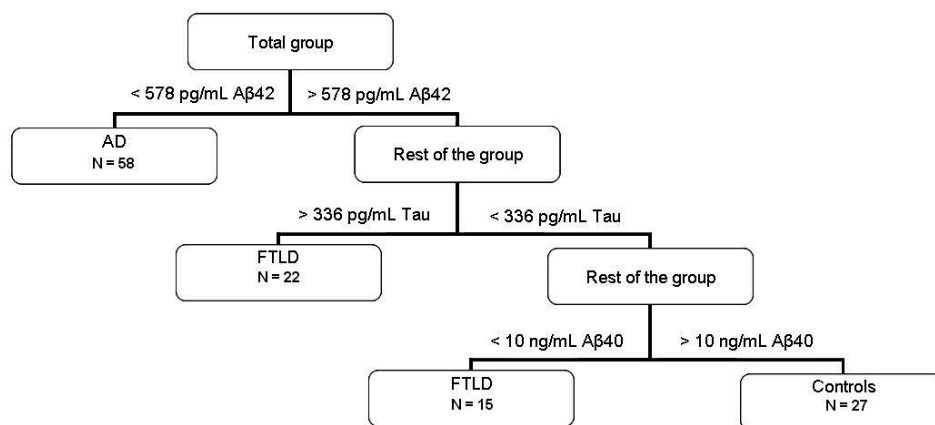


Figure 1. The decision tree model identifies AD patients using CSF A β 42 levels at a cut off value 578 pg/ml (PPV 97%). Subsequently, the FTLD group was selected using CSF tau levels (with a cut-off value 336 pg/ml). Finally, the control group (PPV 68%) was separated from the FTLD group (PPV 67%) by use of CSF A β 40 levels [no difference between the methods used to measure A β 40; cut-off in-house method = 10 ng/ml; cut-off commercial ELISA 6 ng/ml). If only A β 42 and tau were used, the overall PPV was 70% and this was increased by CSF A β 40 levels to 75%.

Table 2. Summary of studies that distinguish controls, AD, and FTLD with a combination of CSF biomarkers.

Study	N		Markers		AD versus FTLD			FTLD versus CON			AD versus CON		
	CON	FTLD	AD		AUC	sens	spec	AUC	sens	spec	AUC	sens	spec
This study	40	55	60	A β 42, tau and A β 40* A β 42 and ptau	---	87%	84%	---	86%	80%	---	95%	95%
Bibl et al. ²⁹	30	30	30	A β 38/ A β 40	---	87%	87%	---	87%	90%	---	100%	97%
Schoonenboom et al. ¹¹	21	28	47	A β 42 and ptau	---	72%	93%						
Bian et al. ⁸	21	28	47	A β 42 and tau							---	81%	100%
Lewczuk et al. ³¹	13	30	19	tau/ A β 42	0.93	79%	97%						
Riemenschneider et al. ⁴	22		35	A β 42/ A β 40							0.95	95%	91%
Kapaki et al. ¹⁵	40	34	74	tau and A β 42	0.89	85%	85%	0.91	90%	77%	0.97	92%	95%
	93	34	76	tau/ A β 42	0.82	77%	81%	0.89	81%	87%			
Shoji et al. ³²	93	34	76	ptau/ Tau							0.98	93%	100%
	34		55	A β 40/A β 42 and tau							---	69%	88%

* Model chooses in-house method.
--- not reported

Since the PFNA group had significant lower CSF A β 42, we omitted these patients from the FTLD group and performed again the logistic regression. A β 42 and ptau-181 remained the best biomarkers subset to discriminate AD patients from FTLD patients (sensitivity 86% and specificity 92%). To discriminate FTLD from controls, levels of CSF tau and the A β 40 were the best combination (sensitivity 82% and specificity 75%). When we used the identified algorithm (=decision tree model), we yield for bvFTD a PPV of 75%.

Comparing the results of A β 40 levels measured by both methods, a strong correlation was found ($r=0.9$; $p<0.01$). The complete statistical analysis yielded the same results for both methods.

DISCUSSION

In this study we investigated the additional value of CSF A β 40 to the existing CSF biomarkers A β 42, tau and ptau-181 in the differential diagnosis between FTLD, AD and controls. We found that CSF A β 40 levels can aid in the discrimination between FTLD and controls and to a lesser extent in the discrimination between AD and controls. The PPV calculated by the decision tree model for A β 40 was only 67%, however, together with the logistic regression analysis, this model confirmed that CSF A β 40 levels help to distinguish FTLD from controls.

FTLD versus AD

No differences in CSF A β 40 levels (measured with both systems) were found between AD and FTLD. This is in contrast with our previous study, investigating smaller patient groups.¹⁰ The finding of higher CSF A β 42 and lower tau and ptau-181 levels in FTLD than in AD is in line with other studies.^{4-11,15} Moreover, a sensitivity of 87% and a specificity of 84% (to discriminate FTLD and AD) is similar to results of earlier studies from our group and others (see Table 2), using various combinations of biomarkers.^{4,8,11,15,29,30}

FTLD versus controls

With the present study we confirmed the presence of lower CSF A β 40 levels in FTLD compared with controls using two separate methods.¹⁰ In addition, higher levels of CSF tau were found in FTLD patients in comparison with controls whereas no differences were found for A β 42 and ptau-181. This is in line with several other studies yielding heterogeneous results, probably due to clinicopathological heterogeneity of the FTLD group itself (Table 2).⁴⁻¹⁵ In this study, the best diagnostic discrimination between FTLD and controls was reached by using A β 42 and tau combined with A β 40 (sensitivity and specificity of 86% and 80%), resulting in an essential higher diagnostic accuracy than the use of CSF A β 42 and tau alone. Until now, only one study applied A β 40 in a model to discriminate FTLD from controls, showing a sensitivity of 87% and specificity of 90% in a model with CSF A β 38.²⁹ As that study used western blot measurements, we cannot compare our results to those for technical reasons.

AD versus controls

As expected, an increase of CSF tau and ptau-181 CSF levels and a decrease of CSF A β 42 levels were observed in AD as compared with controls.¹⁶⁻¹⁸ Similar levels of CSF A β 40 in AD and controls were found, which is in line with our preliminary study,¹⁰ and with others.^{29,31-38} Yet, in this study, the best diagnostic potential, in terms of sensitivity and specificity (both 95%), was reached combining A β 42 and tau with A β 40. This partially covers results found in other studies (see Table 2).^{11,31,32} Shoji and colleagues found, like in the present study, that the accuracy of neurochemical discrimination between AD and controls was improved when A β 42 and tau CSF levels were combined with A β 40 CSF levels.³²

In this study, lower levels of A β 40 have been found in FTLN patients compared to controls. This is quite difficult, since it is believed that in at least half of all FTLN cases a tauopathy with little or no A β is involved.^{39,40} However, several colleagues published evidence which link the tau abnormalities with A β .⁴¹⁻⁴⁴ In line with this, it has been hypothesized that common upstream drivers cause both elevation in A β and tau hyperphosphorylation through independent but parallel mechanisms.⁴³ For example, one of the link-hypotheses concerns functioning of the GSK3 enzyme. GSK3 phosphorylates the tau peptide to form ptau-181 which eventually will cluster to filaments. Additionally, GSK3 interacts with presenilin, which in turn is needed for the gamma-secretase cleavage of APP to create A β .⁴¹⁻⁴⁴

One of the strengths of the present study is that A β 40 CSF levels were measured with two different ELISAs. Group analysis of these two different measurements gave the same results making our conclusions stronger. Overall, lower A β 40 CSF levels were measured with the commercial ELISA. This is probably due to different sets of monoclonal antibodies used in the two tests, or to a different strategy used concerning the N- and C-terminal specificity of the coating and detecting antibodies. Furthermore, different standard preparations used in the two tests may have attributed to the outcome differences. In contrast to our preliminary study, larger numbers of patients have been used in this study increasing the statistical power. There is a statistical limitation, since the AD and FTLN are overrepresented in this study. The baseline probability to classify the different groups is not equal as in clinical practice. This may influence the results calculated by the decision tree. Additionally, postmortem verification of the clinical diagnoses is lacking in the majority of cases, leaving the possibility of misdiagnosis. However, as we made the clinical diagnosis in a multidisciplinary team, and most patients were followed up for at least one year, it is unlikely that we have included a substantial number of misdiagnoses.

If the decision tree is being used in clinical practice, one has to take in consideration that there is a large inter-center variability for the measurements of CSF biomarkers in AD (for A β 42, tau and ptau-181).⁴⁵⁻⁴⁷ This high variability results in differences in reference ranges and reference values, limiting the generalizability of our results. A simple interpretation algorithm to compare biomarker results between centers, as reported by Lewczuk and colleagues, can be helpful in this perspective.⁴⁸

In summary, although the role of CSF A β 40 appears to be limited in the distinction between FTLN and AD, our findings demonstrate an additional value of A β 40 in the discrimination of FTLN patients from non-demented subjects.

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